Integration of Water Treatment and Water Analysis Technology
- Introduction of AIST Water Project -

Hiroaki TAO

AIST Shikoku Center, National Institute of Advanced Industrial Science and Technology (AIST)

Today's Presentation

- Introduction of AIST
- Outline of AIST Water Project
- R&D on Water Measurement Technology
- R&D on Water Treatment Technology


National Institute of
Advanced Industrial Science
and Technology
AIST
AIST Tsukuba

AIST Hokkaido

Bioproduction Research Institute
Methane Hydrate Research Center

AIST Tohoku

Research Center for
Compact Chemical System

AIST Shikoku

Health Research Institute

AIST Chugoku

Biomass Refinery Research Center

Fukushima Renewable Energy Institute

Renewable Energy Research Center

AIST Tsukuba Headquarters

▸AIST Tsukuba

Twenty-eight research units are located at AIST Tsukuba

AIST Tokyo Headquarters

AIST Tokyo Waterfront

Molecular Profiling Research Center for Drug Discovery
Digital Human Research Center
Center for Service Research

AIST Chubu

Advanced Manufacturing Research Institute
Materials Research Institute

AIST Kyushu

Measurement Solution

Employees

[foreign nationals]

Researchers	**2,281 [80]**
●Permanent	2,010
●Fixed term	271
Administrative employees	657
Total number of employees	**2,938 [81]**
Executives	13
Visiting researchers	156
Postdoctoral researchers	259
Technical staff	1,602

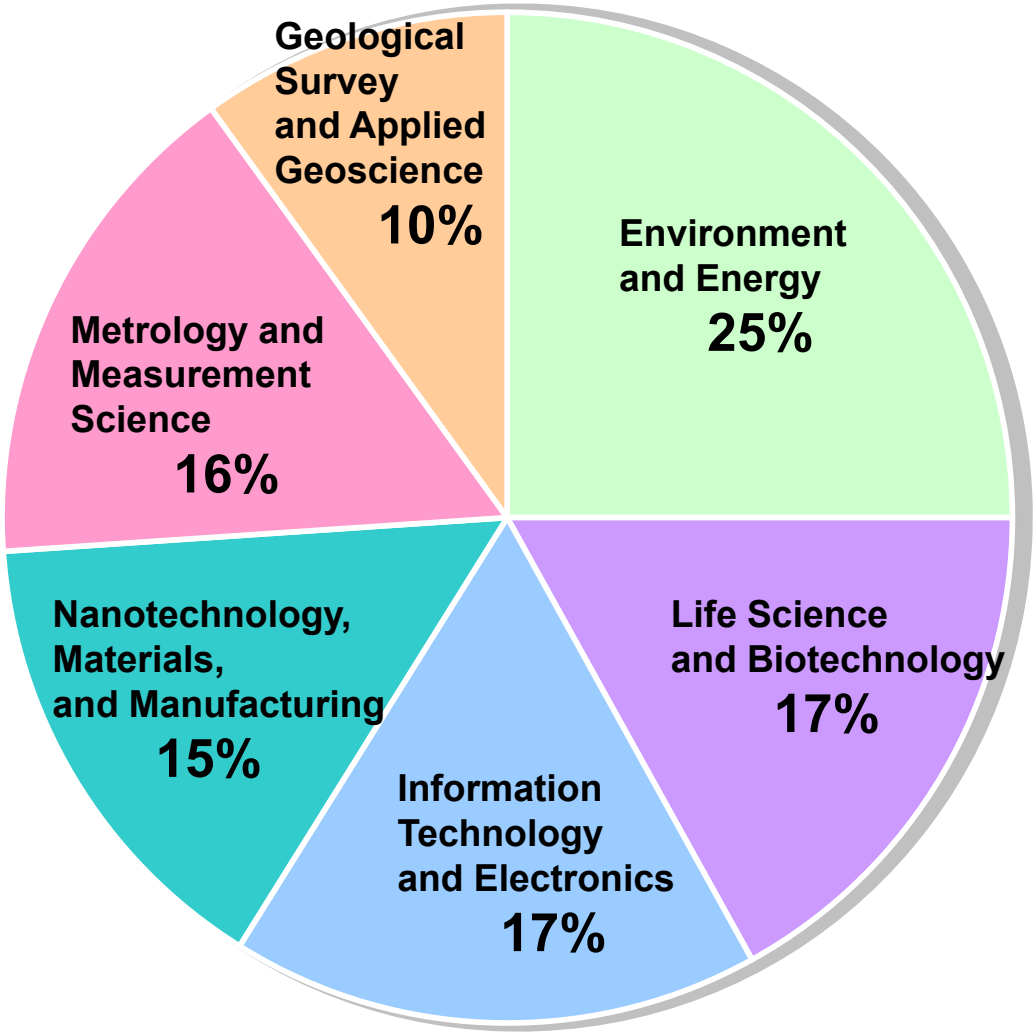
Researchers accepted through industry-academia-government partnerships

【400 from overseas】

From companies	Approx.1,700
From universities	Approx.2,000
From other organizations	Approx. 800

(NOTE : Total no. of researchers accepted in FY 2012)

Work Force



Composition of research staff by research field

(As of May 1, 2013)

Today's Presentation

- Introduction of AIST
- Outline of AIST Water Project
- R&D on Water Measurement Technology
- R&D on Water Treatment Technology

National Institute of Advanced Industrial Science and Technology
AIST

Ryoji Chubachi,
President of AIST

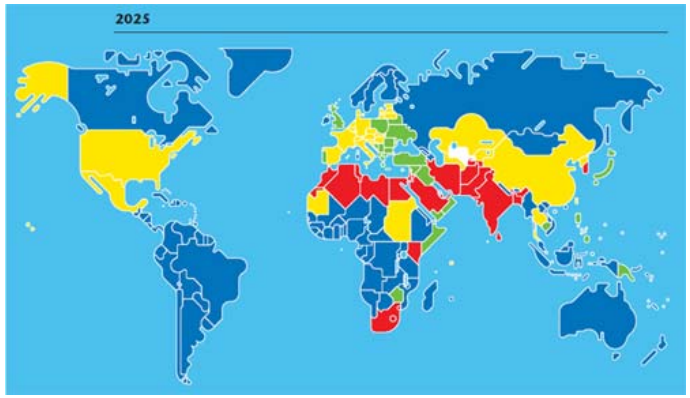
AIST Water Project

Environment and Energy | Life Science and Biotechnology | Information Technology and Electronics | Nanotechnology, Materials and Manufacturing | Metrology and Measurement Science | Geological Survey and Applied Geoscience

- Research Institute for Environmental Management Technology
- Research Institute for Innovation in Sustainable Chemistry
- Health Research Institute
- Biomedical Research Institute
- Electronics and Photonics Research Institute
- Information Technology Research Institute

Dr. Tao
Director of AIST
Shikoku Center

Over 30 researchers of 6 Research Institutes participate in this AIST Water Project



Global water stress
* WBCSD Water Scenarios to 2025

National Institute of Advanced Industrial Science and Technology
AIST

AIST Water Project

AIST Technologies

Overcome the water problems by integrating AIST owned technologies listed below.

- Online water quality monitoring technology
- Highly sensitive heavy metals detection technology
- High-speed microbial image acquisition and processing technology
- Microbial separation & identification technology
- Membrane bioreactor (MBR)
- Decomposition & sterilization technology by photocatalyst
- Various materials for adsorption film
- Water data transfer and processing using cloud technology

Global Collaboration

- Promotion of personnel exchanges and joint researches with the National Institutes and universities in Asia
- International cooperation for standardization of water technologies

Today's Presentation

- Introduction of AIST
- Outline of AIST Water Project
- R&D on Water Measurement Technology
- R&D on Water Treatment Technology

Item to be monitored for risk assessment

1. Index for Organic Pollutants (BOD, COD, TOC)
2. Heavy Metals
3. Physiologically Active Substances (Endocrine Disrupting Chemicals, PPCPs)
4. Whole Effluent Toxicity (WET)
5. Microorganism
6. Multidrug Resistance Gene (New Delhi metallo-β-lactamase)
7. ・・・・

Maintenance-free TOC monitor using photochemical reaction

- Generation of oxidative species, ·OH, with UV irradiation (<190 nm)
- Persistent substances such as humic acid are decomposed to CO_2 in less than 1 min
- No hazardous reagent and heating device

Principle

Lamp-pass-through reactor

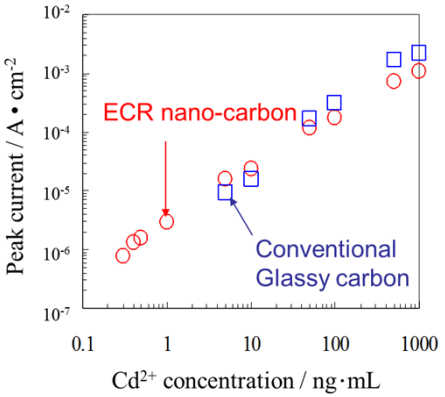
$H_2O + h\nu\ (<190\ nm) \rightarrow H\cdot + OH\cdot$

$OH\cdot + \text{Organic matter} \rightarrow CO_2$

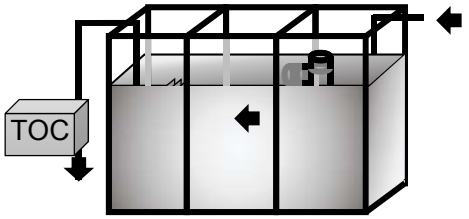
Prototype

TOC, heavy metals

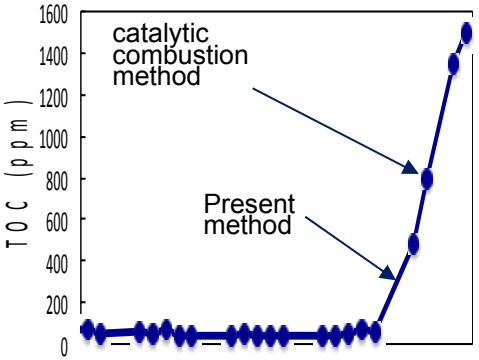
stripping voltammetry using ECR nano-carbon electrode



Continuous Monitoring of MBR



MBR

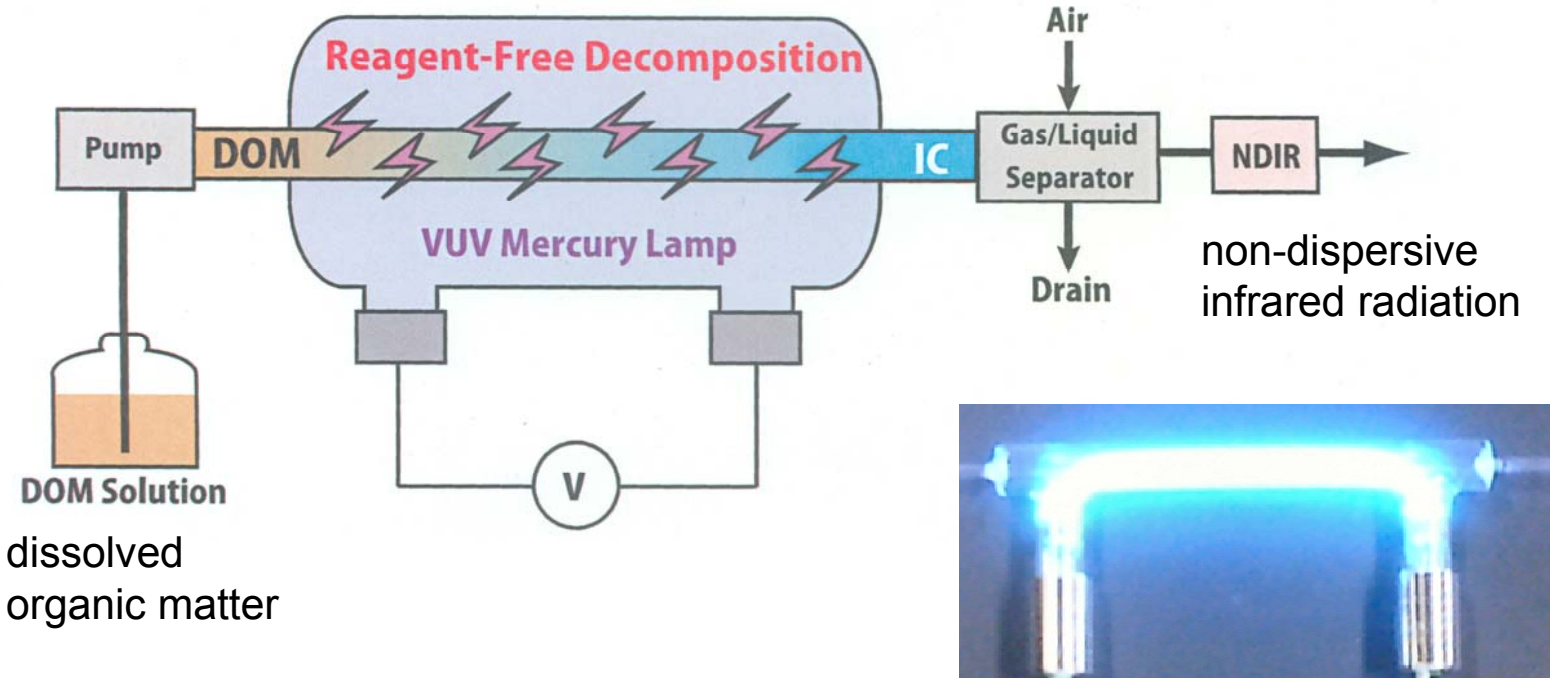


Period: 1 month
Precision :1.8% for 12 h
Good comparability with catalytic combustion method

Comparison with other methods

	present	catalytic combustion method	UV oxidation with $K_2S_2O_8$
reagent	none	Pt catalyst	$K_2S_2O_8$
heater	none	>600°C	~100°C
Detection limit	6.2 μgC/L	48 μgC/L	-
Mainte-nance	no need	exchange of catalyst, cleaning of heater	supply of reagent

Online TOC analyzer using a vacuum UV mercury lamp-pass-through photoreactor

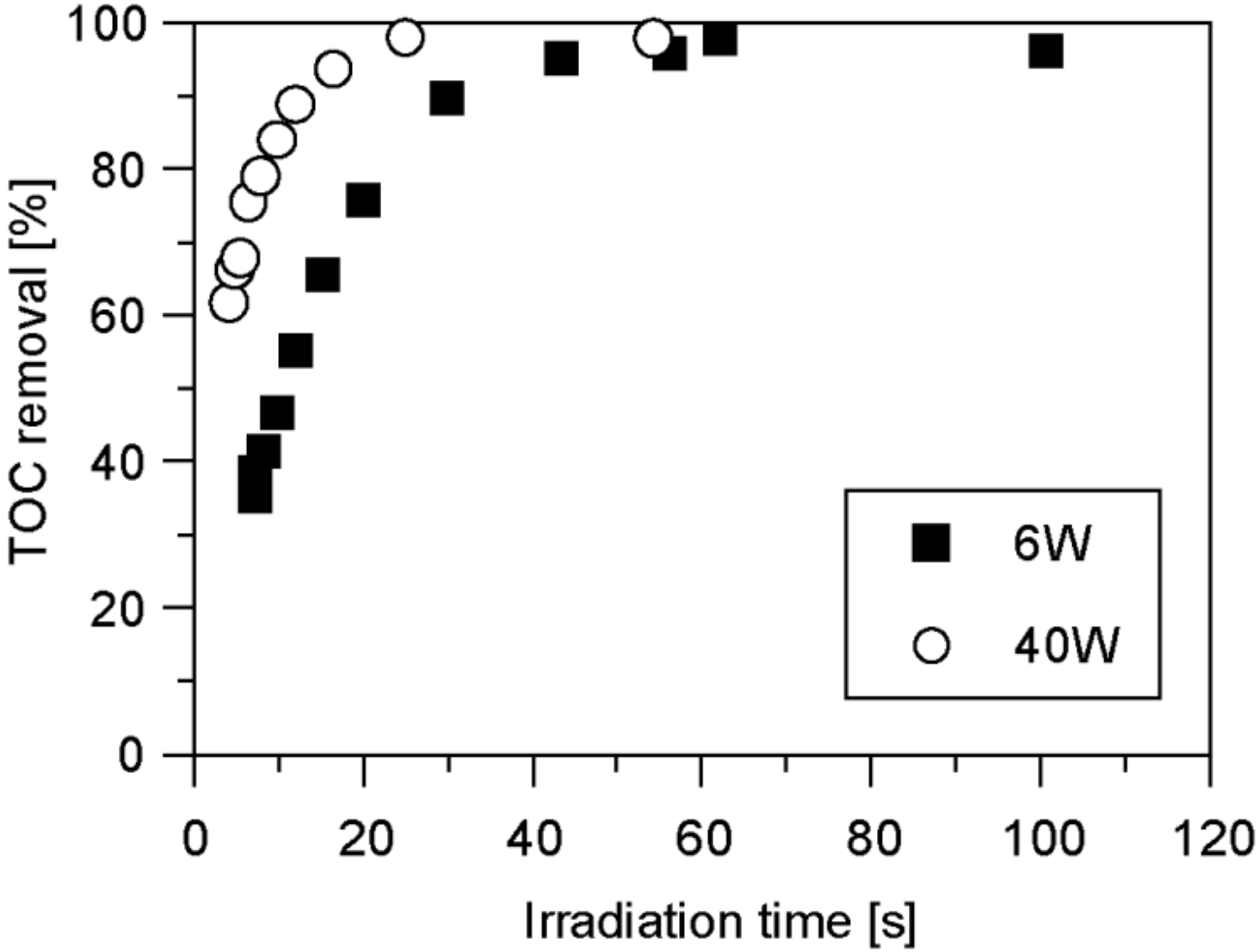


$H_2O + h\nu\ (<190\ nm) \rightarrow H\cdot + OH\cdot$

$OH\cdot + Org.Comp. \rightarrow CO_2$

without oxidizing reagent ($K_2S_2O_8$)

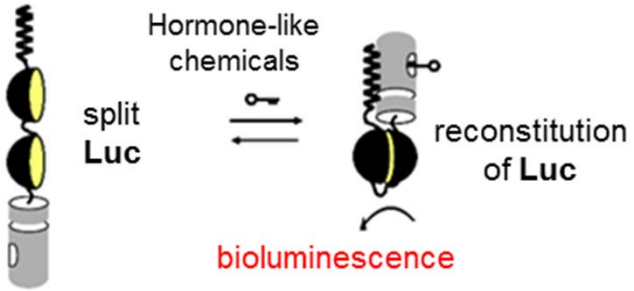
Dependence of lamp output power on the decomposition of fulvic acid at 2 mgC/L



Bioluminescent assay for endocrine-disrupting chemicals

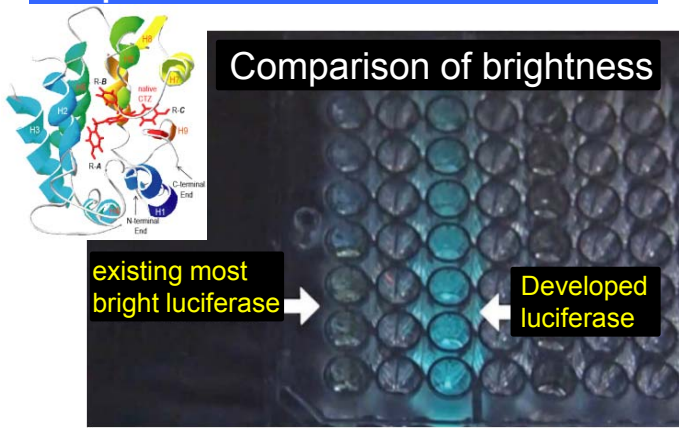
- Hormone receptor-mimicking bioluminescent probes for endocrine-disrupting chemicals (EDCs)
- High-throughput illumination of the activities of EDCs in wastewater and living subjects
- Superluminescent luciferases for supporting a high-sensitive analysis of EDCs of the probes

Principle



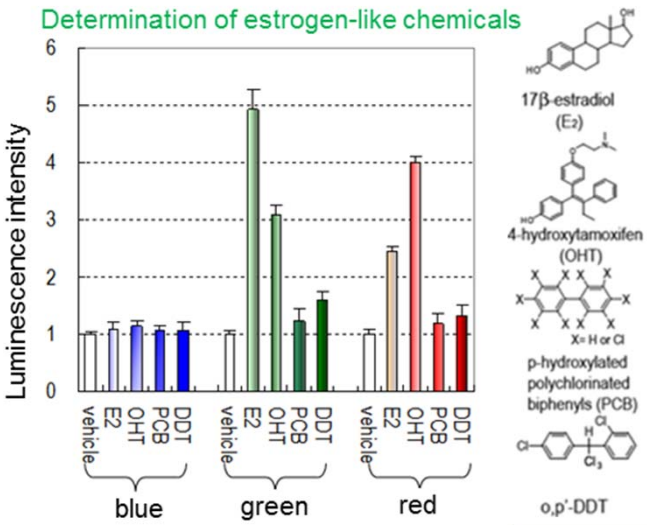
single-chain bioluminescent probe mimicking hormone receptors, which sense hormones and change the conformation. This change exerts reconstitution of the fragments of a luciferase and specific light emission.

Superluminescent luciferase



- 100 times brighter than ever existing luciferases
- half-life period of luminescence is long (20 min)

Determination of estrogen-like activities of chemicals

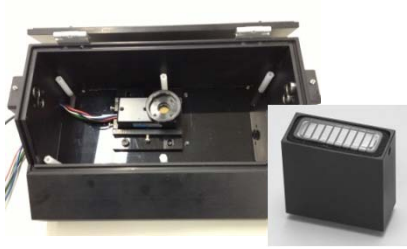


A multicolor imaging probe set. Green and red colors indicate genomic and nongenomic activities of chemicals, respectively.

Step toward the practical use

Cooperation with several companies

Prototype

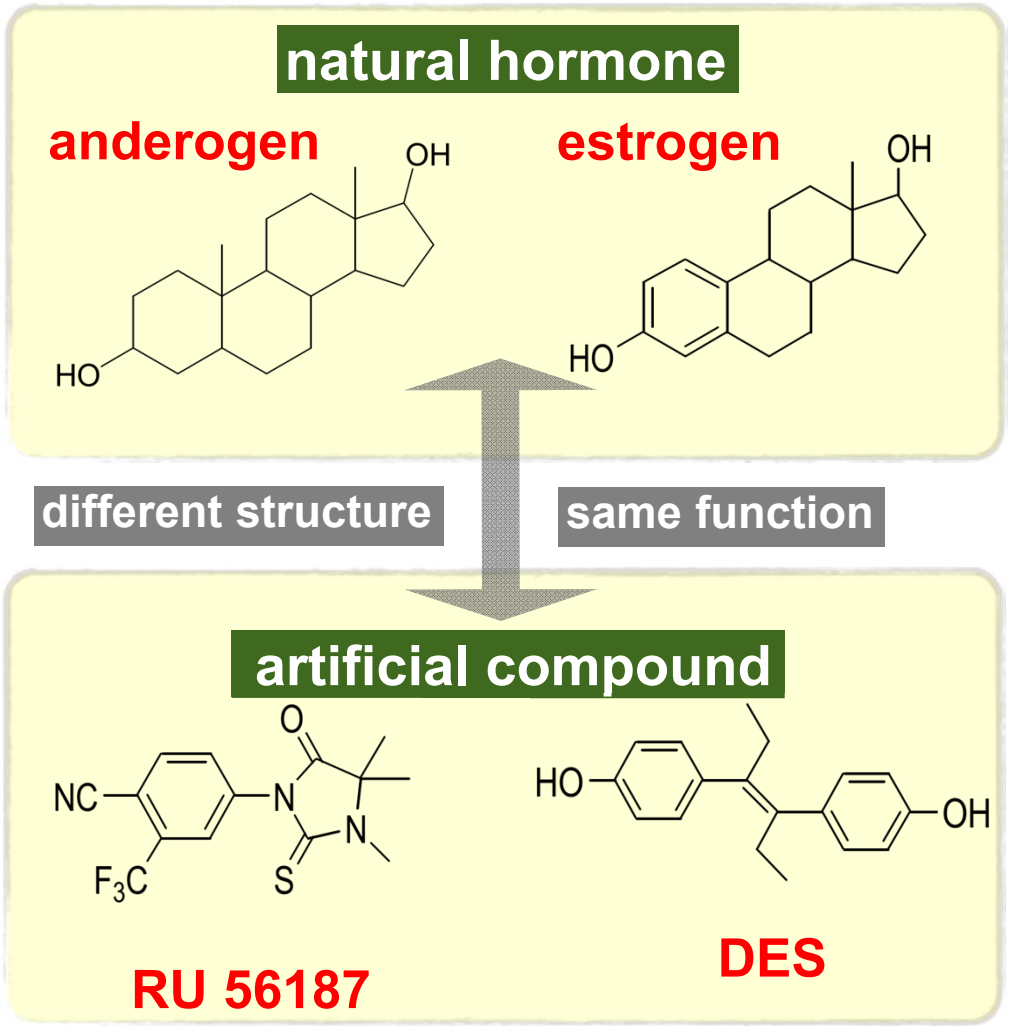


Ongoing research

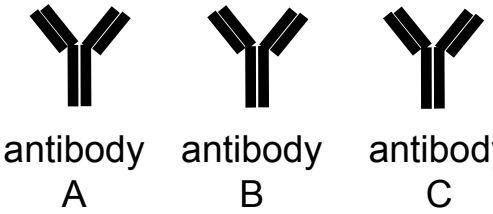
- Application to PPCPs (pharmaceutical & personal care products)
- On-site monitoring
- Evaluation of efficiency of water treatment

Assays for endocrine-disrupting chemicals

endocrine-disrupting chemicals

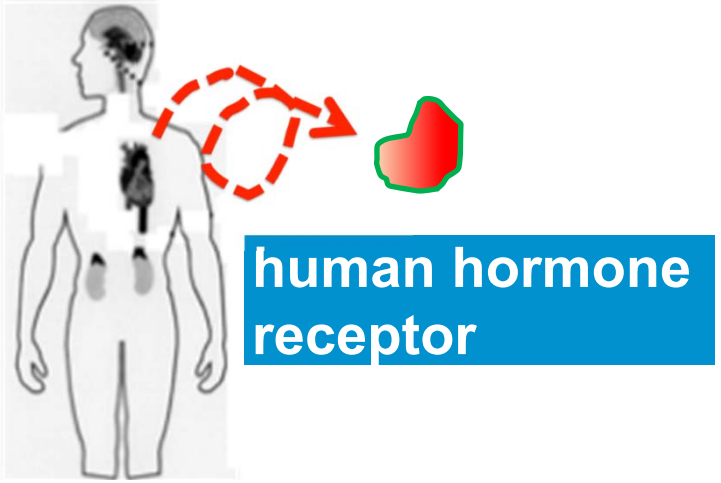


antibody immunoassays

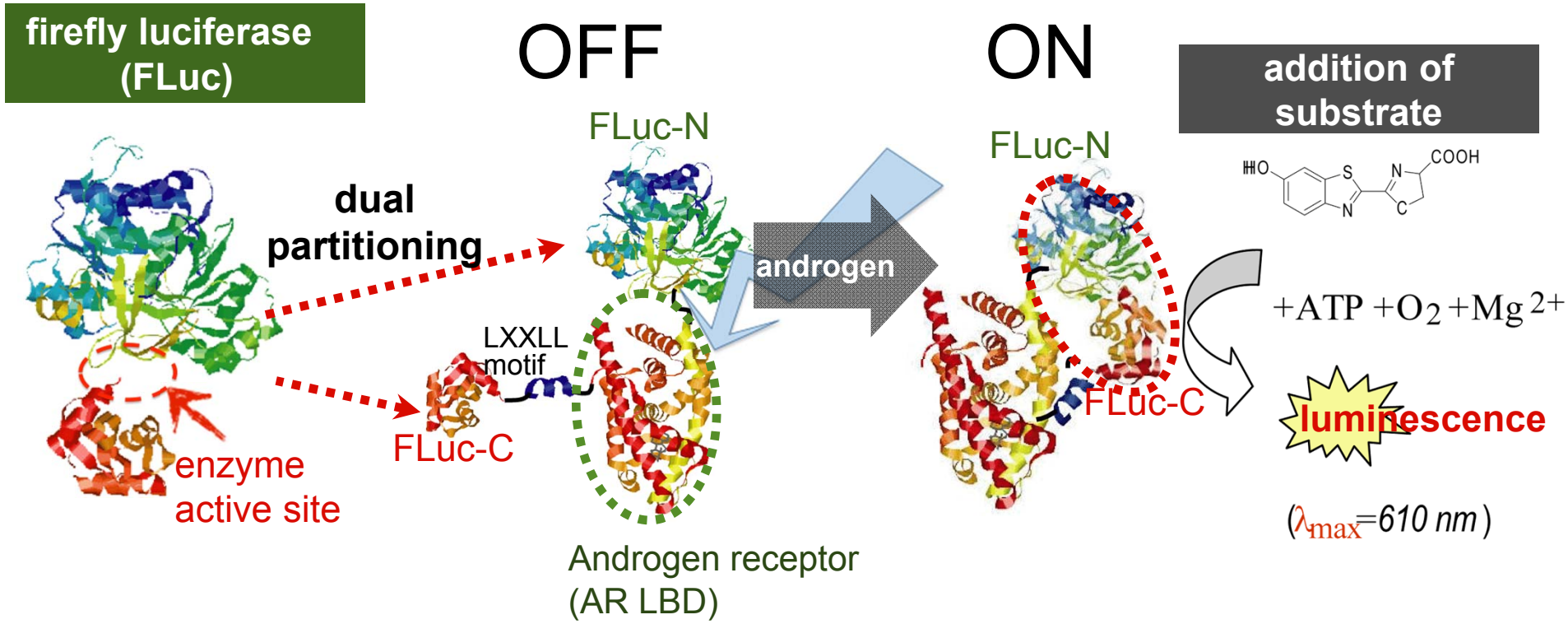


1. high-cost/prolonged time（3h）
2. unavailable to unknown compounds

receptor assays



Principle of Bioluminescent Probe



advantages:

1. Rapid analysis (3h → 20 min)
2. High signal to noise ratio (50 times)
3. Available both in cell system and in non-cell system (test paper)

Development of Superluminescent Luciferase（ALuc™）

Introduction of mutation into luciferase

-ATG AAG TGA-

200 AA x 20 ⇒1/4000
Low success probability

Total synthesis of optimal DNA based on bioinformatics

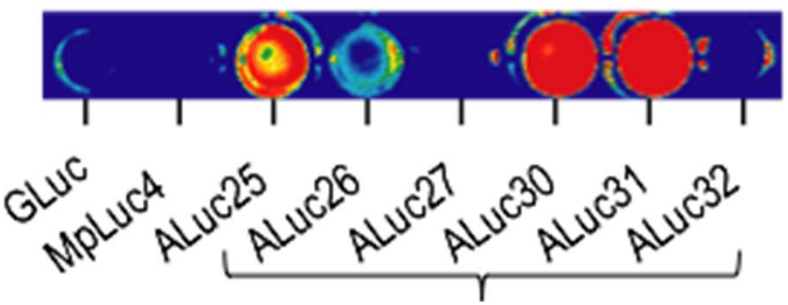
Artificial Luciferase

ALuc™

Comparison of brightness

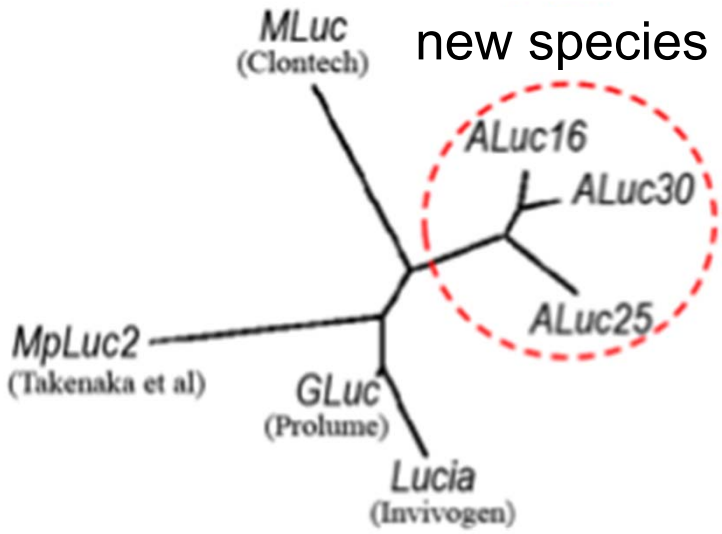
existing brightest luciferase

superlumi nescent luciferase



■ 100 times brighter than ever existing luciferases
■ half-life period of luminescence is long (20 min)

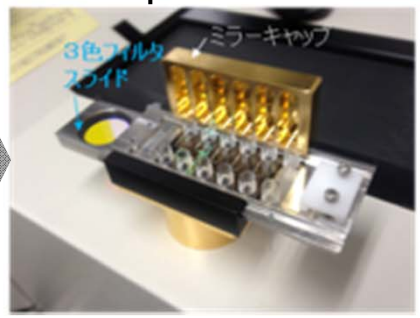
DNA gene tree



Development of portable luminometer

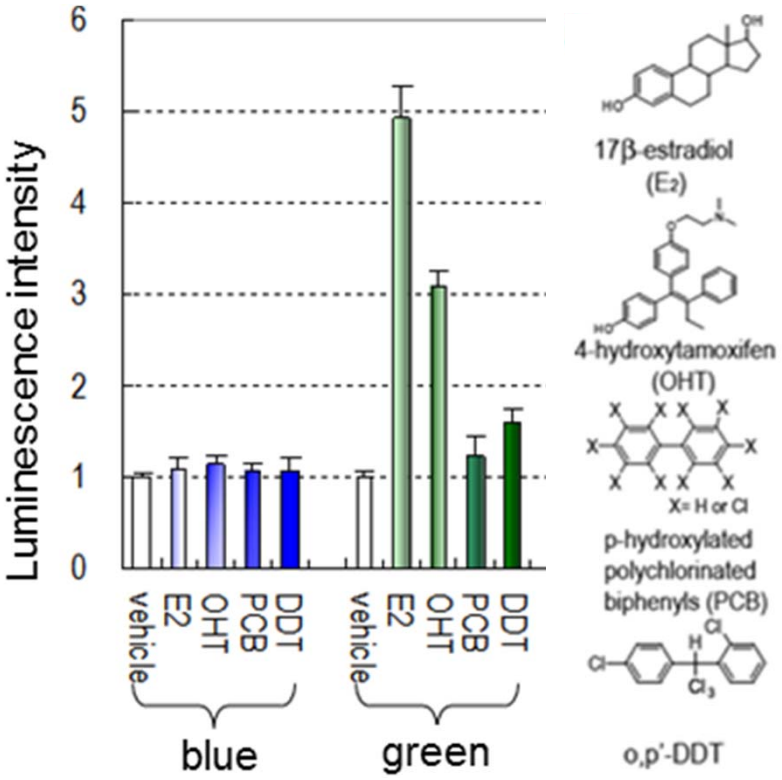
sample tube

optical unit



6 channel photodetector

Determination of estrogen-like activities of chemicals



to be applied to wastewater and treated water by MBR

Biosensor of Hazardous Compounds, based on Microbial Responses

Existing methods for warning water pollution

- Breeding carp and killifish ➡ Check the death on next day
- Microbial sensor（Fuji Electric・Toshiba in Japan）
  ➡ Difficult management of biological sensors

Needs

- Immediate warning system for water pollution ➡ on-site sensor
- Evaluation of Individual compound ➡ WET: Whole Effluent Toxicity
- Acute effect ➡ Chronic effect

Whole cell biosensor for ecotoxicity testing using human iPS cells

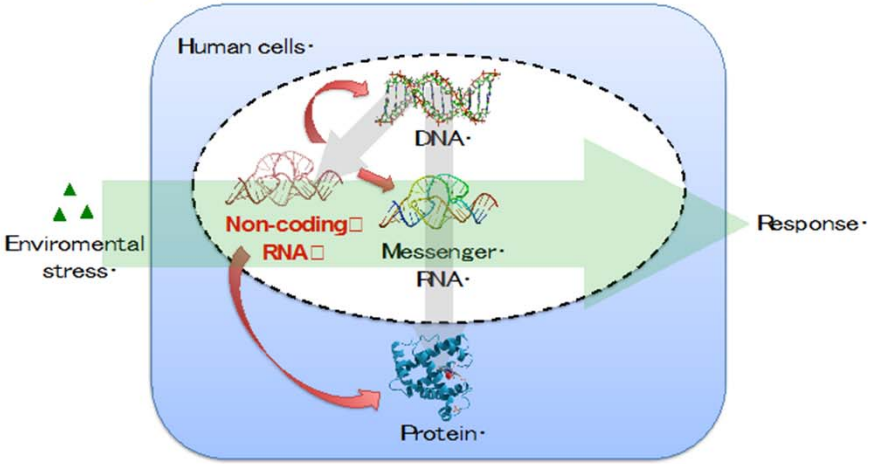
- Whole cell biosensor with broad substrate responses using human iPS cells
- Highly-responsive cell-based assay utilizing non-coding RNAs
- Rapid and cost-effective method for toxicity testing using microdevices

Non-coding RNAs are RNA molecules that are not translated into proteins. Recent transcriptomic and bioinformatic studies indicate that the thousands of non-coding RNAs exist, and newly identified non-coding RNAs dynamically regulate the gene expression in mammalian cells.

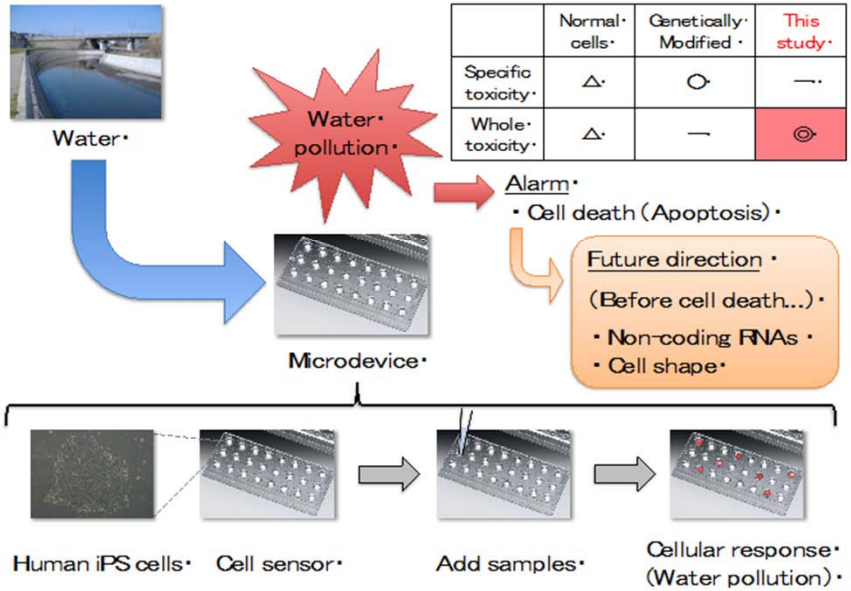
We hypothesized that non-coding RNAs highly respond to environmental stresses, such as ecotoxicological substrates. We have developed a highly susceptible cells that died by environmental stresses faster than normal cells. This technique is capable of rapid and sensitive method for toxicity testing.

We focus on the human iPS cells that can differentiate into various cells and tissues. In the future, we will assess the ecotoxicity of environmental samples to each human tissue using human iPS cells. Moreover, we will developed a rapid and cost-effective devices for ecotoxicity testing.

Importance of non-coding RNA in human cells



New sensor devices using human iPS cells



Detection of microorganisms using optical disk and image recognition technologies

- Early detection without culturing microorganisms
- Rapid scanning and low cost system using optical disk
- Identification of the organisms by image recognition and DNA probe hybridization techniques

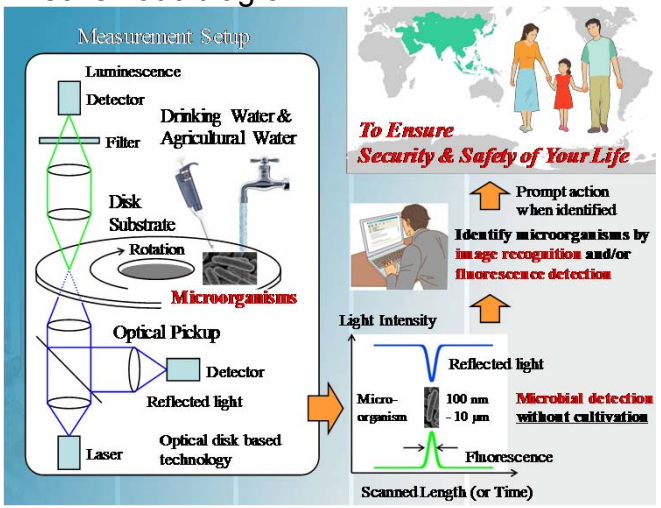
Research target

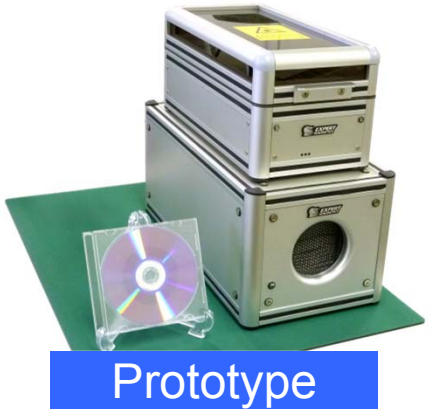
Detection and identification of pathogenic microorganisms such as *Escherichia coli* within several hours without cultivation by combined use of our original technologies including optical disk techniques and image recognition based on HLAC (Higher-order Local Auto Correlation).

Research content

We measure the light intensity from an optical disk when microorganisms are attached and rebuild an image of the microorganisms by scanning many grooves and aligning the results properly. We roughly identify microorganisms from the cell shapes by pattern recognition techniques based on HLAC. We also use DNA probe hybridization technique for more accurate identification of microorganisms based on the fluorescently-labeled probe.

A schematic diagram





Prototype

Comparison with other techniques (Advantages are shown in red.)

	Optical Microscope	Optical Disk	Cultivation (e.g., *E. coli*)
Recognition of the attachment	Just after the measurement		Cultivation time: > 1 day
Identification	Simple test within several hrs		# 1-3 days for simple test # 5 days for definitive test
Scanning time (Area: 100 cm^2)	22 hrs (2 s /img. @40x†)	0.2 hrs (@DVD 6X)	———
Expertise	High	Low	Extremely high
Apparatus cost	$10k - $50k	$50k (initial stage) $100 (as of DVD)	$30k - $50k
Inspection cost	$1 - $2 (Glass sub.)	$1 - $2 (Polycarbonate sub.)	$20 - $50‡

$: in US dollars, †: an assumption with no automatic-scanning stage,
‡: depending on with or w/o the employment costs

Detection of Microorganisms in Water Using Optical Disk and Image Recognition Technologies

AIST

Measurement Setup

Fluorescence

Detector

Filter

Drinking Water &
Agricultural Water

Disk
Substrate

Rotation

Microorganisms

Optical Pickup

Detector

Reflected light

Laser

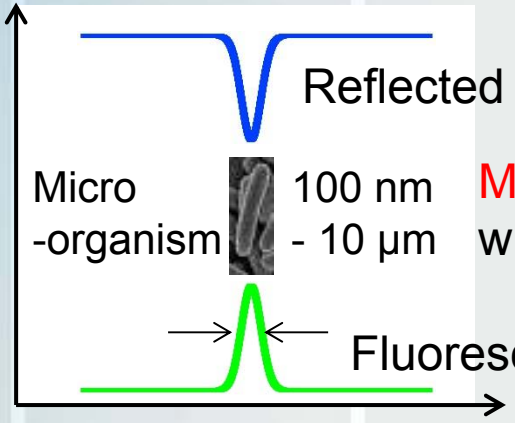
Optical disk based
technology

To Ensure
Security & Safety of Your Life

Prompt action
when identified

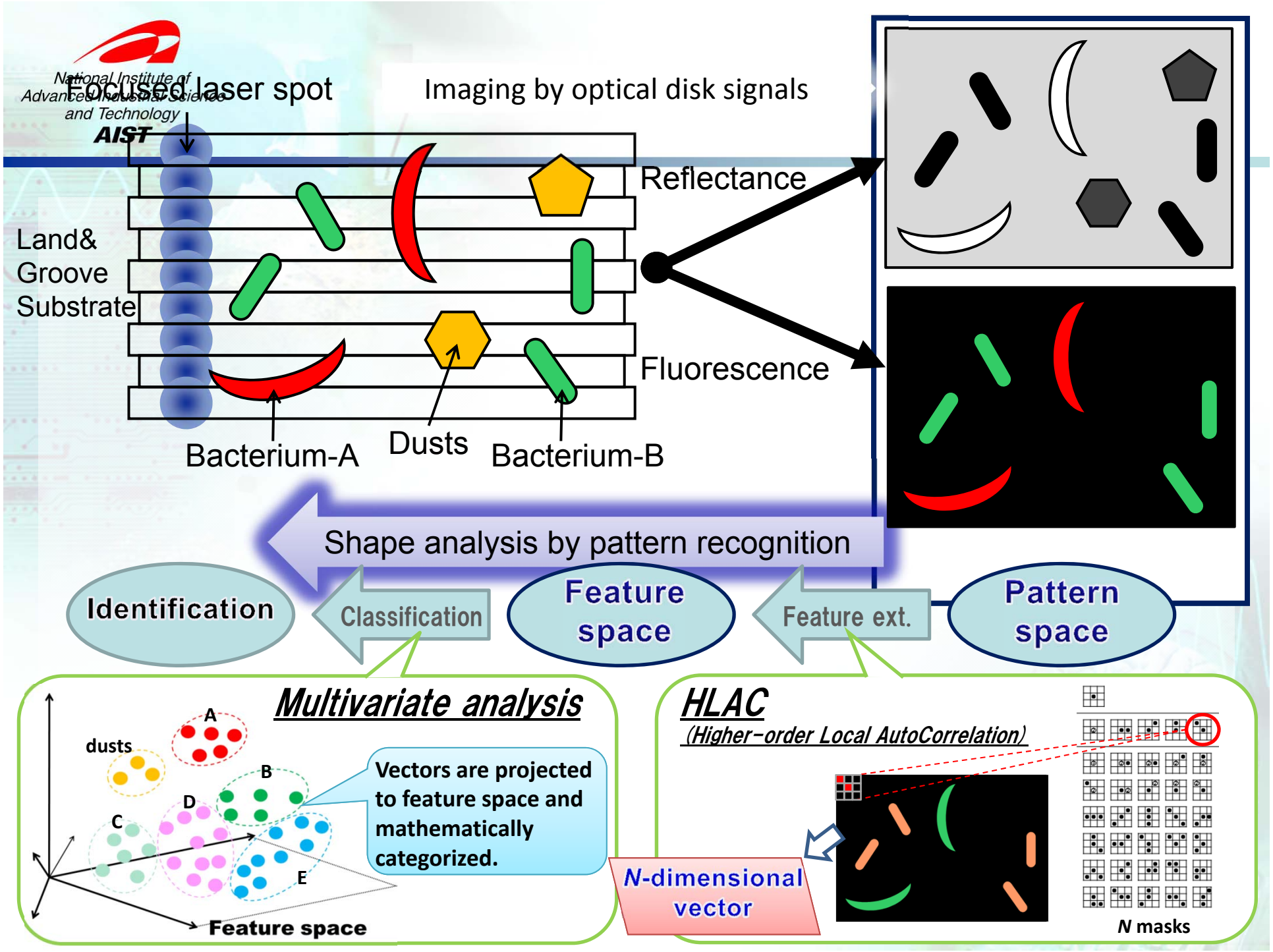
Identify microorganisms by
image recognition and/or
fluorescence detection

Light Intensity



Microbial detection
without cultivation

AIST


National Institute of Advanced Industrial Science and Technology
AIST
Focused laser spot
Imaging by optical disk signals
Land&
Groove
Substrate
Reflectance
Fluorescence
Bacterium-A
Dusts
Bacterium-B
Shape analysis by pattern recognition
Identification
Classification
Feature space
Feature ext.
Pattern space
Multivariate analysis
A
dusts
B
D
C
E
Feature space
Vectors are projected to feature space and mathematically categorized.
HLAC
(Higher-order Local AutoCorrelation)
N-dimensional vector
N masks

	Optical Microscope	Optical Disk	Cultivation (e.g., *E. coli*)
Recognition of the attachment	Just after the measurement		Cultivation time: > 1 day
Identification	Simple test within several hrs		# 1-3 days for simple test # 5 days for definitive test
Scanning time (Area: 100 cm^2)	22 hrs (2 s /img. @40x†)	0.2 hrs (@DVD 6X)	———
Expertise	High	Low	Extremely high
Apparatus cost	$10k - $50k	$50k (initial stage) $100 (as of DVD)	$30k - $50k
Inspection cost	$1 - $2 (Glass sub.)	$1 - $2 (Polycarbonate sub.)	$20 - $50‡

$: in US dollars, †: an assumption with no automatic-scanning stage,
‡: depending on with or w/o the employment costs

AIST

Optical disk sensor (prototype)

Optical disk drive
(Measurement)

Signal processing unit
(Analysis)

EXPERT MAGNETICS

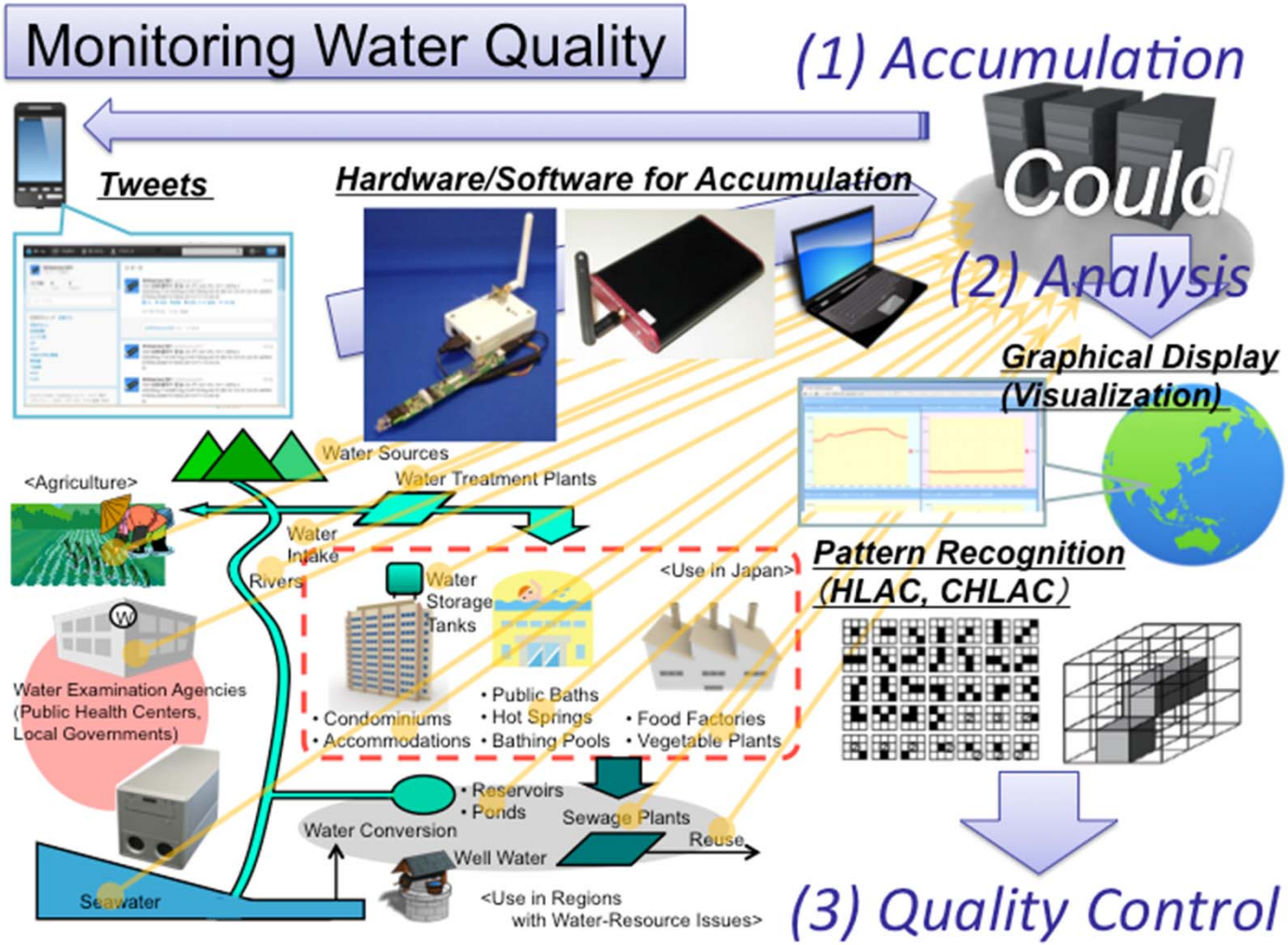
AIST

National Institute of Advanced Industrial Science and Technology
AIST

Smart Sensor Network for Environmental Management

- Cloud-based water quality monitoring & controlling sensor network system
- Both simple measurement using SNSs and large-scale analysis using cloud are possible
- Also applicable to power consumption management and home security system

The water management system is a large-scale distributed system which monitors and controls water resource, and its key to high reliability and low cost is utilization of the cloud computing. We have developed several hardware and software combining various sensors and the cloud systems, and have realized both simple and easy monitoring using social network services such as Twitter® and large-scale information accumulation and analysis using cloud services such as Google App Engine®.



Today's Presentation

- Introduction of AIST
- Outline of AIST Water Project
- R&D on Water Measurement Technology
- R&D on Water Treatment Technology

Membrane bioreactor (MBR)

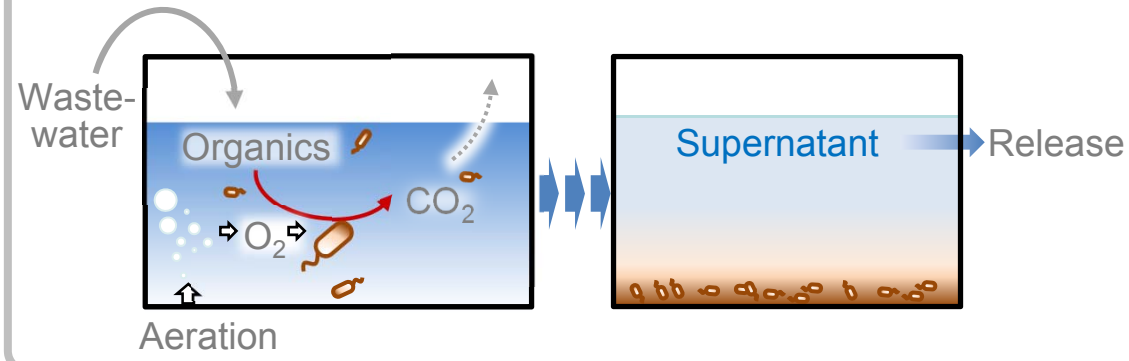
Wastewater treatment process;

Combination of membrane process and activated sludge (AS)

Conventional

Degradation

Precipitation



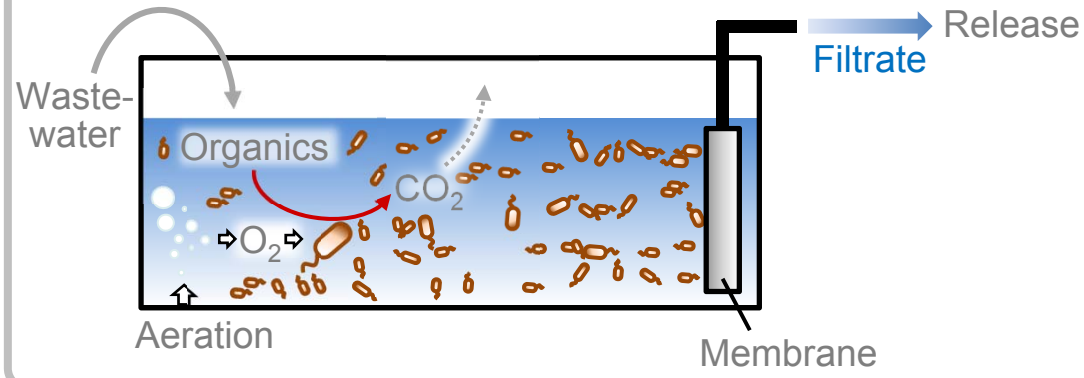
Drawbacks

- Precipitation requires
  - a large ground area
  - a long time
  - control of AS conditions

MBR

Degradation

Filtration

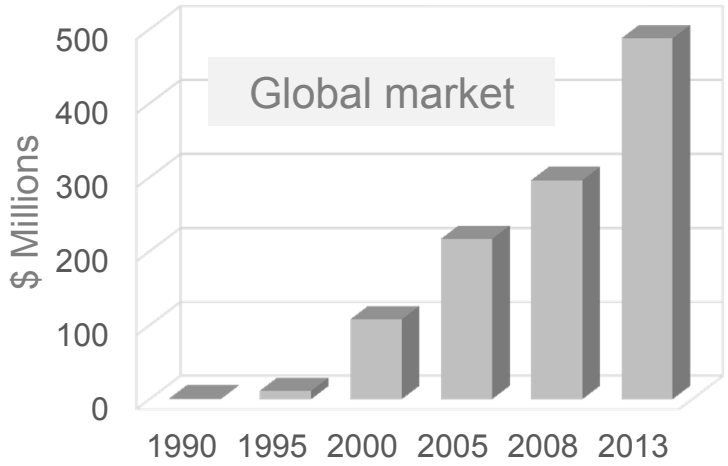


Advantages

- Small space
- High density microbes degrade organics at higher rates

Current concerns of MBR operation

- Market size is growing
  ⇒ Becoming more important



(Prof. TorOve Leiknes (2011): Key elements and bottlenecks of the membrane bioreactor (MBR) process for advanced wastewater treatment, CSWEA Education Seminar)

http://www.cswea.org/events/2011edseminar/

- MBR requires experienced operators
  ⇒Deal with case-by-case problems

Indicators of reactor condition are required for stable performance

Objectives of this study

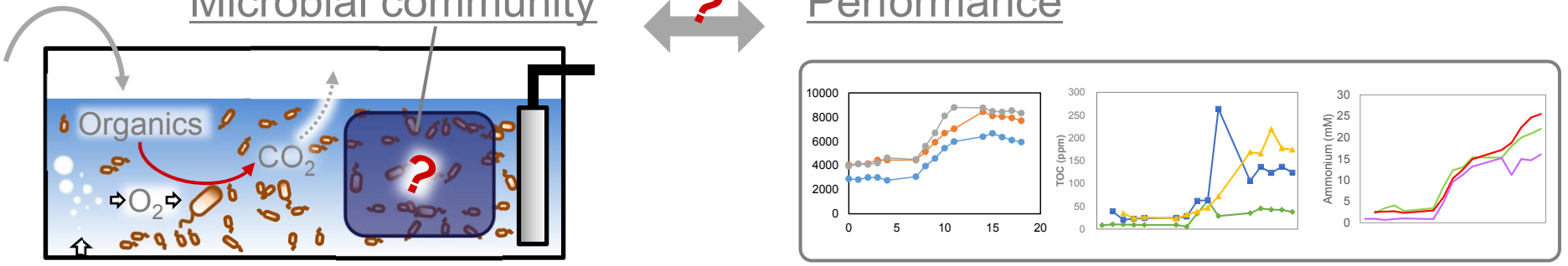
Make criteria for MBR operation not empirically dependent

(1) Under optimum performance

⇒ How microbial community changes?

⇒ How the community relates to performance?

Microbial community ? Performance

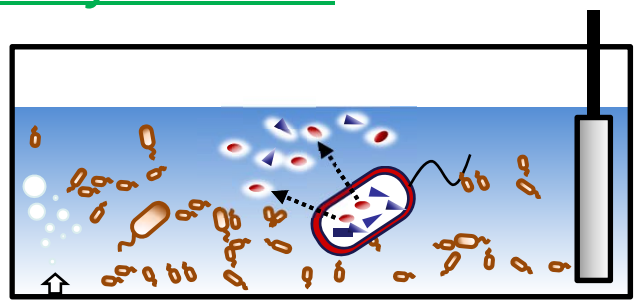


Investigate microbial community changes at high resolution by next-generation sequencing

(2) Identification of microbes requires long analysis time

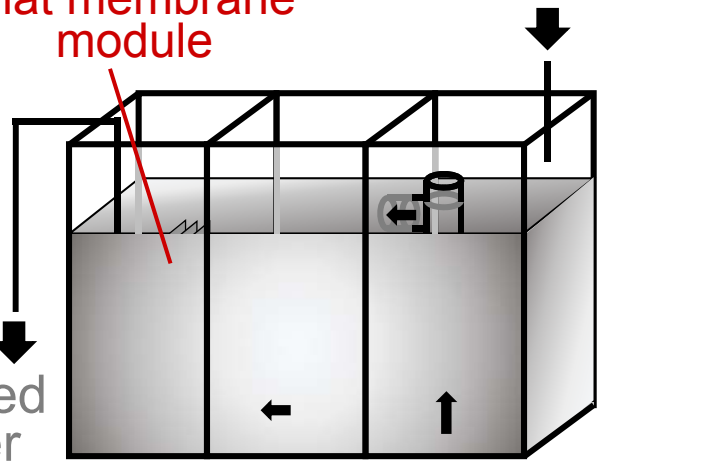
⇒ Any other indicator?

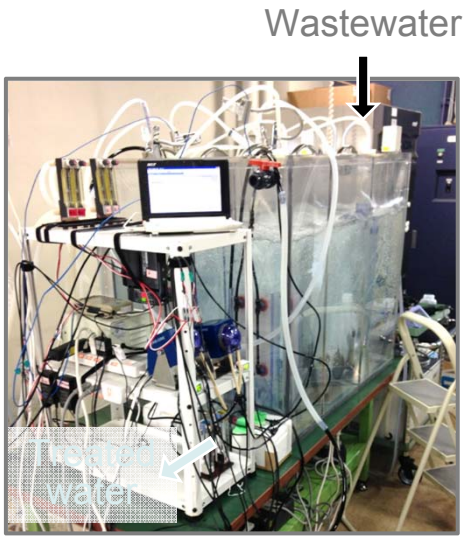
Focus on supernatant proteins in MBR



Design and operation of pilot scale MBR

Pilot scale MBR





Flux

- *Volume* — *230 L*
- Wastewater (in) — 115 L/day
- Treated water (out) — 115 L/day
- *HRT* — *2 days*

Organic loading change [TOC]

1130 mg/L: first 1 week

2260 mg/L: following 2 weeks

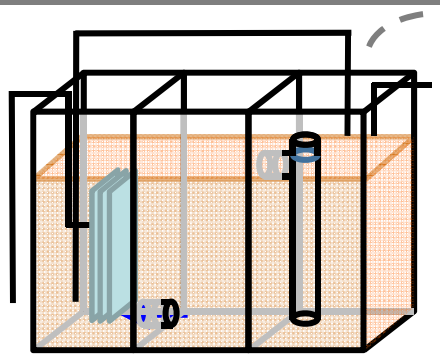
Measurements

- *MLSS*
- *COD*
- *TOC*
- TN
- Amines (*NH_3*, DMA, TMA)
- DO
- Flow rate, Transmembrane pressure
- *Microbial community* …etc.

Organic loading was increased by 2 times during the operation

⇒Link the reactor performance to microbial community

Next-generation sequencing (NGS) analysis



Activated sludge

c.f.

DNA extraction, PCR (16S rRNA gene),
purification, quantification

Illumina MiSeq

Data/run

- Total yield: ~ 15 Gb
- Sequence length: 300 b × 2
- Read number: ~ 50 millions
- Sample number: ≈ 200 by using tags

↓ Sequence data analysis

↓ Sequence join (ea-utils), q30 quality selection (qiime),

↓ chimera seq remove (mothur), phylogenetic analysis (qiime)

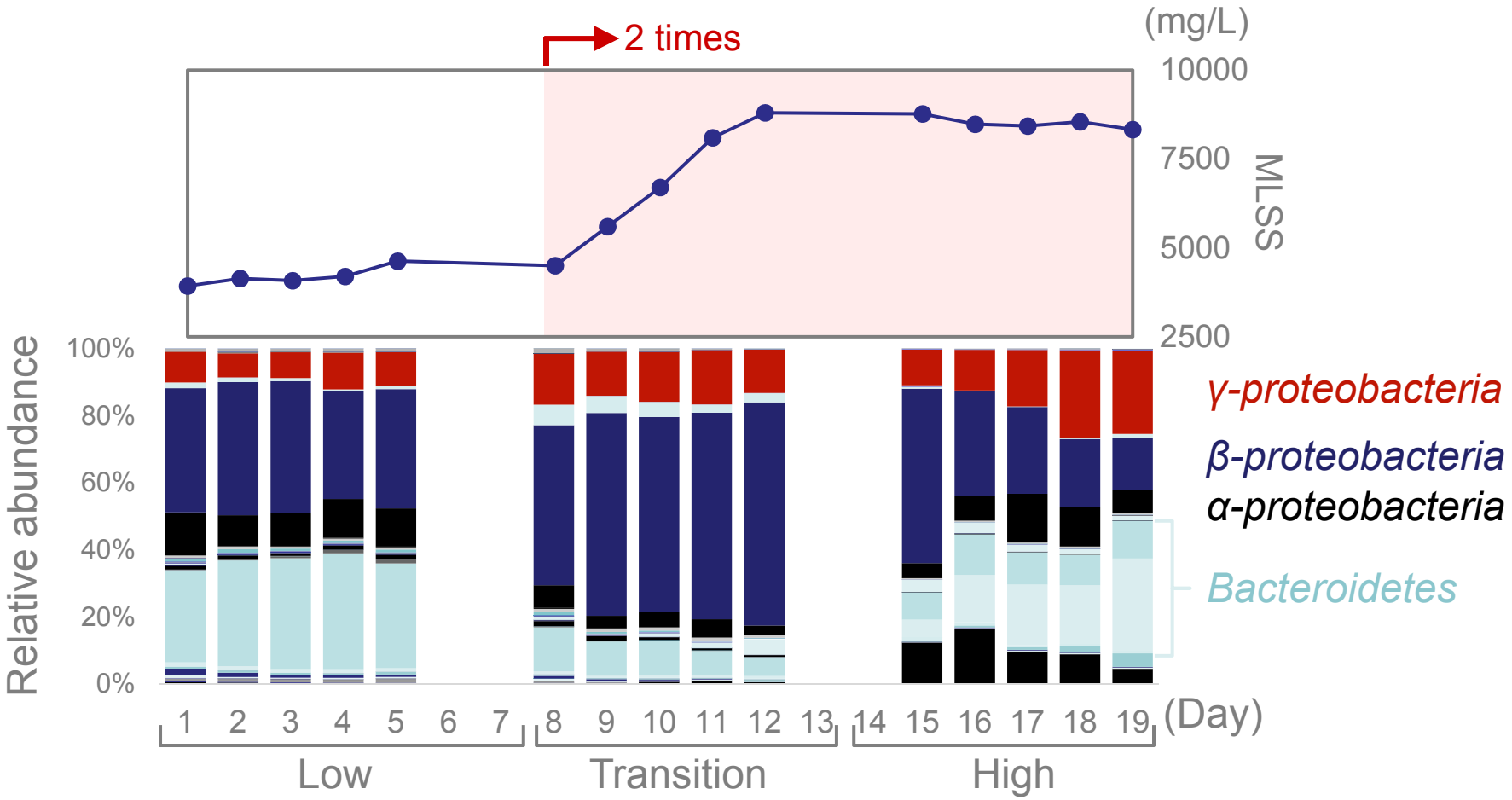
This method identifies millions of species/run

⇒30,000~60,000 reads/sample

Changes of microbial community at Class-level

All sludge samples were analyzed by NGS

⇒Classified at "**Class**"-level



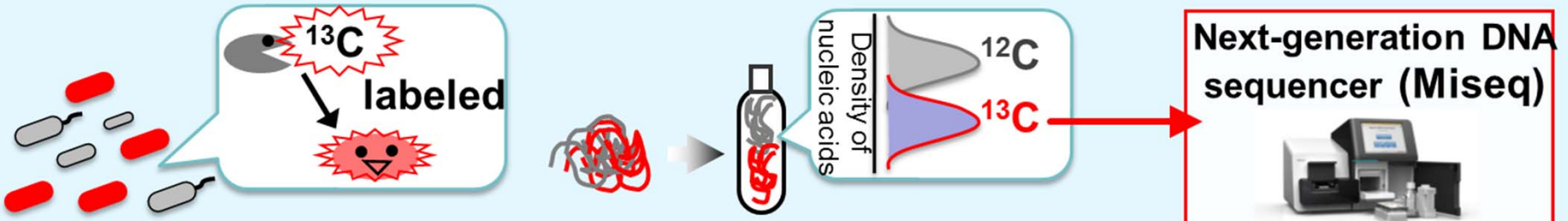
Under high organic loading conditions

⇒Microbial community was drastically changed

⇒*γ-proteobacteria and Bacteroidetes became dominant*

Stable isotope probing (SIP)

A powerful tool to identify the function of uncultured microbes



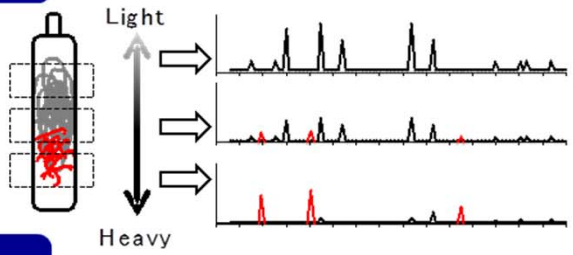
● Cultivation of microbes with stable isotopes

● Nucleic acids extraction & density gradient centrifugation

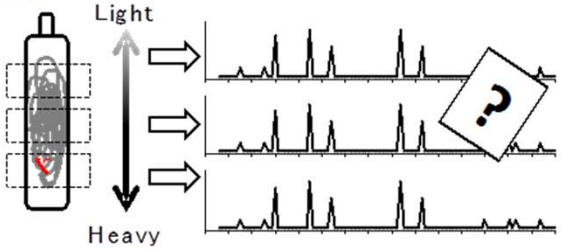
● High sensitive detection of the labeled microbes

Conventional

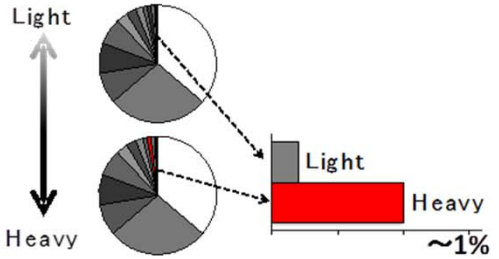
T-RFLP fingerprinting



Advanced



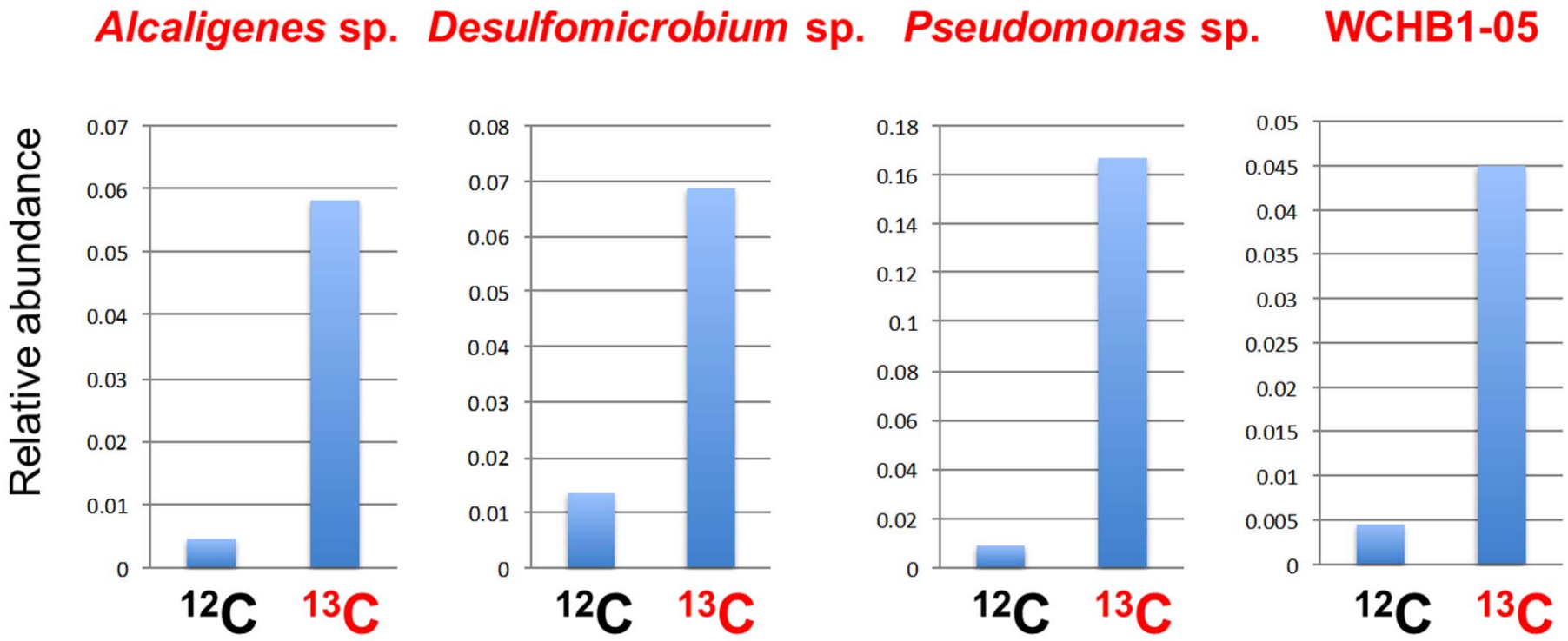
16S deep sequencing



Combined with deep sequencing

A slight portion (0.001%) of ^{13}C-labeled RNA was identified phylogenetically

^{13}C-incorporating microbes: Oil degraders



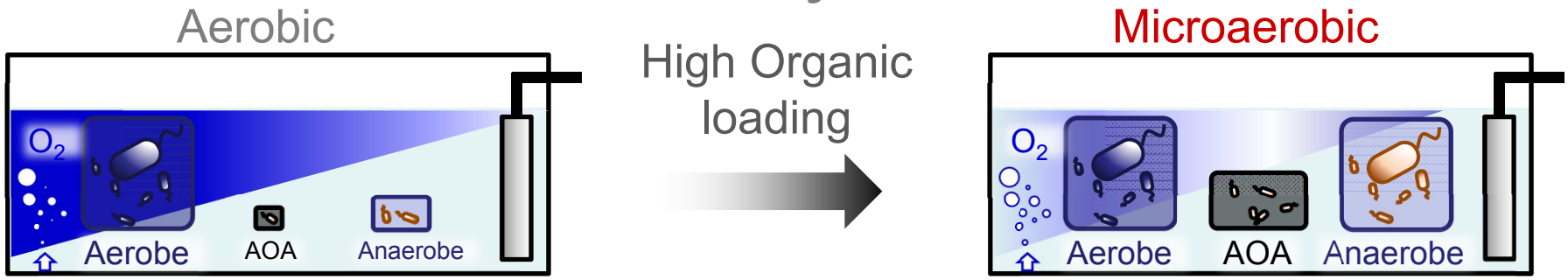
- **Four species of microbes were accumulated in the heavy fraction**

→ **These were involved in degradation of palmitate, (an oil component)**

A combination of SIP and deep sequencing enabled the sensitive identification of ^{13}C-assimilaing microbes

Summary of this presentation

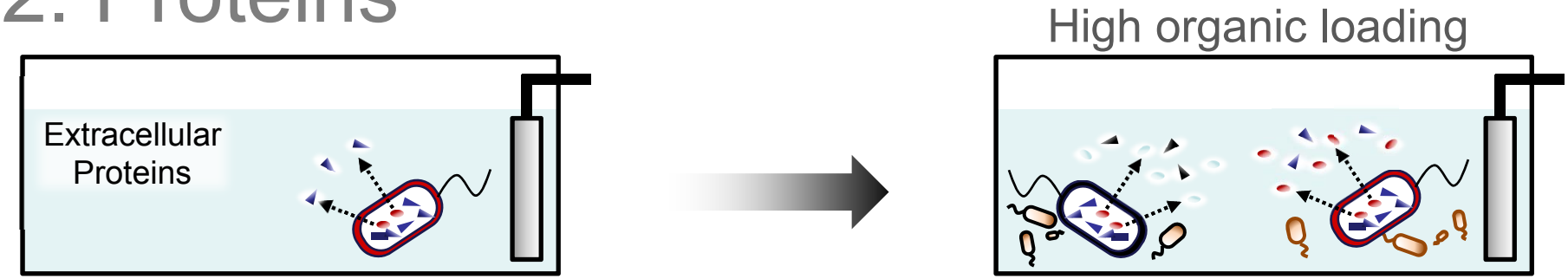
1. Microbial community



Under high organic conditions,

- Anaerobes and related species were highly increased
- Coexistence is important

2. Proteins



Under high organic conditions,

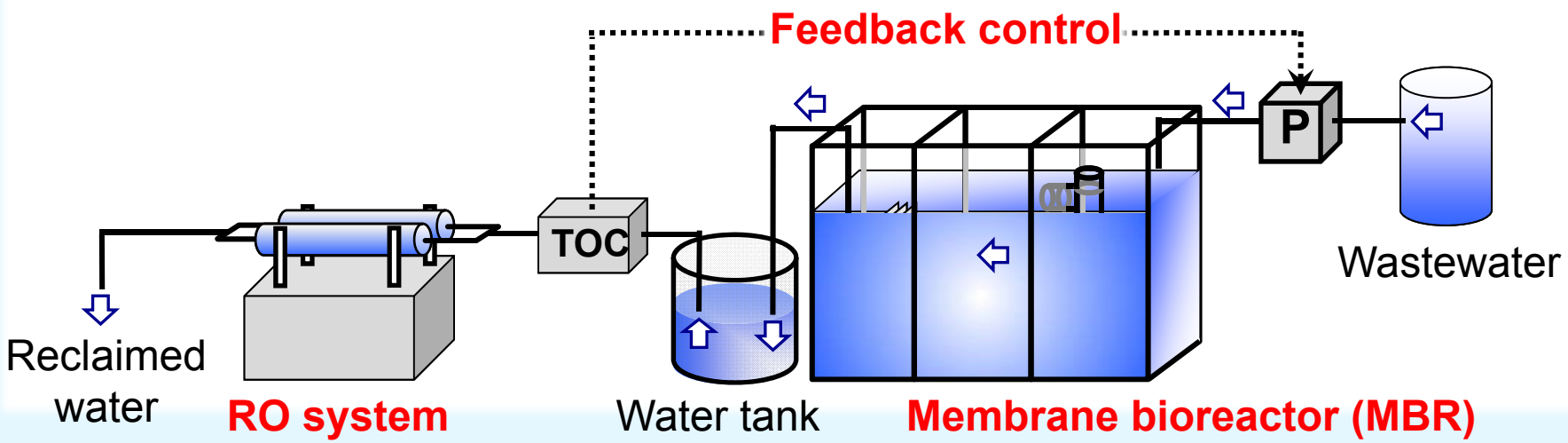
- Several proteins were found, and correlated to the conditions
- They can be used as indicators ⇒ Continue to analyze

Summary (MBR)

- **Construction and operation of the pilot-scale MBR**
- **Demonstration of the microbial community dynamics in the MBR with high resolution using next-generation DNA sequencer**
- **SIP combined with 16S rRNA deep sequencing successfully identifying the key players in the activated sludge**

NEXT FOCUS: MBR and RO system

1) Stable and efficient control of microbial community by continuously monitored physicochemical parameters.
2) Biofouling mechanism of membrane by physicochemical and microbial analyses.



Nanocomposite for Cleaning Environmental Pollutants

●Novel hybrid nanostrucutres of carbon nanosheets (1 to tens graphene layers) and metal / metal oxide nanoparticles achieving synergy of adsorption and catalysis

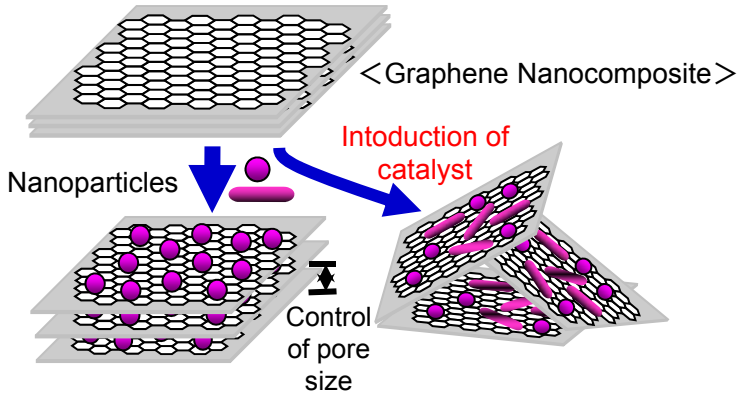
Research target

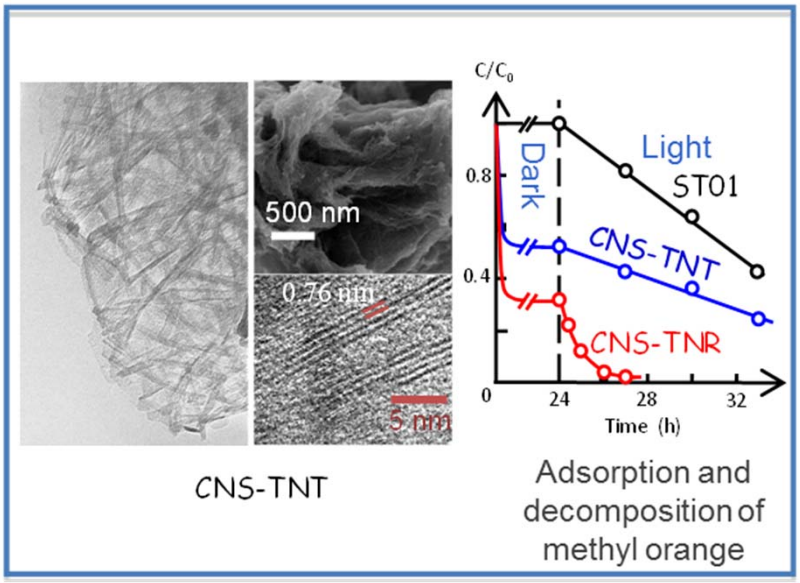
Aiming at efficient removal of trace chemicals (POPs, PPCPs, etc.) which have potential impacts on human beings and ecosystem, we are developing technology for nanostructured composition of graphene (G) and metal/metal oxides. Nanocomposite from titania and G demonstrates a high efficient adsorption concentration-induced photocatalysis.

Research content

By combining intercalation and hydrothermal methods, a synthesis technique which utilizes carbon nanosheets (CNS) as a template for 2D deposition of 1D titanate nanotube (TNT) or nanorod (TNR) was developed. Through adsorption concentration of the substrate CNS, photoactivity of the composite was promoted by 5 to 6 times as compared to the pure titania.

Nanocomposite achieving synergy of adsorption and catalysis





Water purification by solar power

Research target

- Drinking water purification in developing country
- Research of photo catalytic system adjusted to the local area
- International collaboration

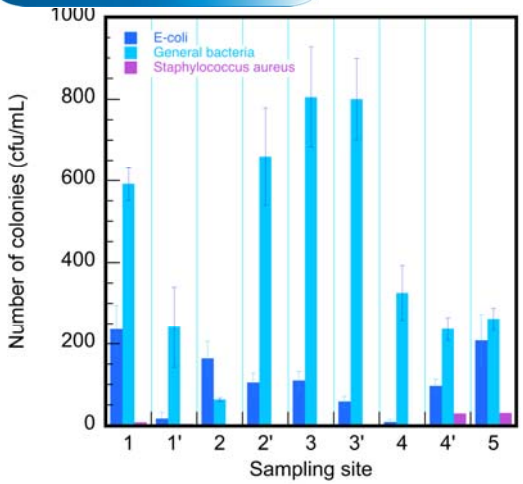
カレン族住宅
河川水を直接導引

リス族集落
日本から援助された陶器壷で雨水を飲用として貯留

タイ族集落
援助団体の資金で井戸を掘削

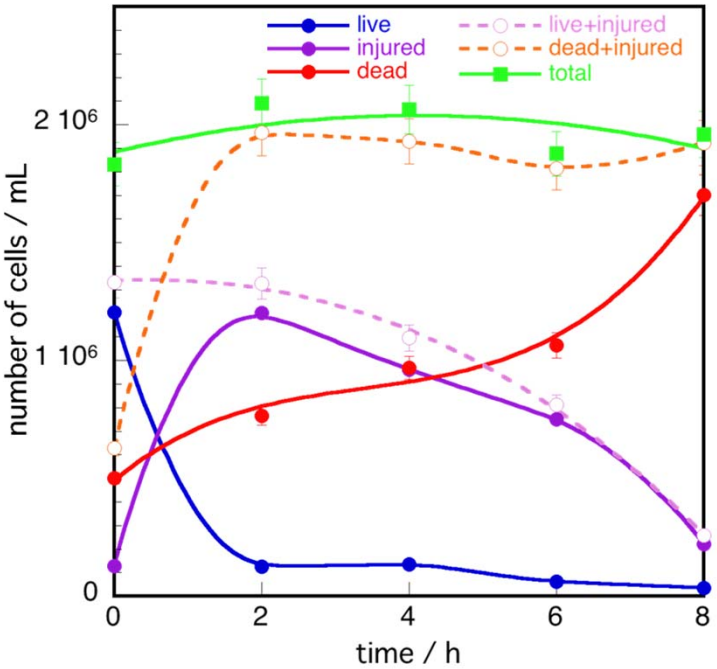
タイ・チェンライ県におけるサンプリング調査
（山岳民族とでは生活レベルに大きな差）

Research content



１：ポンパケーム村（ヤオ族）導水管
１’：ポンパケーム村（ヤオ族）導水をペットボトルに詰めたもの
２：ジャコナ村（ラフ族）導水管
２’：ジャコナ村（ラフ族）導水を金属容器に貯留したもの
３：ヘーコー村（リス族）導水をペール缶に貯留したもの
３’：ヘーコー村（リス族）雨水（非飲用）
４：チャンジャーサイ村（アカ族）導水管
４’：チャンジャーサイ村（アカ族）導水をペットボトルに詰めたもの
５：カレアンコヨー集落（カレン族）貯水池からの導水管

タイ･少数民族集落（チェンライ県）の飲料水並びに生活用水中の大腸菌並びに一般細菌群。WHO基準から見ると飲用不可レベル。



E.Coli treatment results. 1x10⁶ cfu/mL of E.coli was successfully disinfected within 8 hours. (1L)

Current Collaboration

Vietnam
VAST
JSPS Bilateral Programs
MBR, Photocatalyst

Thailand
TISTR, NSTDA (NANOTEC)
Field Experiment for Sterilization of Drinking Water

China
Research exchange
Tsinghua Univ., Sichuan Univ.
Nano-materials for Water Treatment

Future Collaboration

Indonesia (national institutes companies and universities)
Singapore
India